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Termitomyces sheikhupurensis sp. nov. (Lyophyllaceae, Agaricales) from Pakistan, evidence from morphology and DNA sequences data

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Abstract: A new symbiotic species from genus *Termitomyces*, is proposed here supported by morphological features and molecular evidence. The species is characterized by an annual growth, pileus with velar remnants, ruptured margins, pubescent stipe with short pseudorhiza, basidia varying in wall thickness, polymorphic cheilo- and pleurocystidia as well as two types of pileipellis hyphae. Sequences of nr ITS and LSU regions of newly reported species nested as a separate taxon in both phylogenetic analyses of current study.

Keywords: Lyophyllaceae, new species, Pakistan, Hiran Minar, sclerobasidia, subhumid

1. Introduction

In 1942, a group of gilled agarics that live in a mutualistic relationship with termites, named *Termitomyces* was introduced (Heim, 1942). *Termitomyces* species belong to the family Lyophyllaceae, are recognized by the termite association, their fleshy agaricoid fruiting bodies, pluteoid carpophores, often with a sharp conspicuous umbo, free to adnexed lamellae though with decurrent tooth stipe, subterranean pseudorhiza connected to termite nest, (few species are deficient in this characteristic). Anatomically, *Termitomyces* species have typically ellipsoid, smooth, inamyloid basidiospores, cystidia present, tramal system monomitic, hyphae inamyloids, with or without clamp connections (Heim, 1942, 1977; Pegler, 1977; Frøslev et al., 2003; Wei et al., 2009; Aryal and Budathoki, 2015; Aryal et al., 2016; Tang et al., 2020).

The genus is widely distributed from southern and southeastern Asia to all sub-Saharan regions, America, India, Pakistan, Cameroon, Indonesia. (Heim, 1942; Otieno, 1964; Alasoadura, 1966; Pegler and Rayner, 1969; Pegler 1977; Batra and Batra, 1979; Natarajan, 1979; Moriss, 1986; Van der Westhuizen and Eicker, 1990; Pegler and Vanhaecke, 1994; Gómes, 1995; Ahmad et al., 1997; Aanen et al., 2002; Aanen and Eggleton, 2005; Aanen, 2006; Wei and Yao, 2003; Tibuhwa et al., 2010; Nobre et al., 2011; Karun and Sridhar, 2013; Anwar et al., 2020; Njouonkou et al., 2020; Sathiya Seelan et al., 2020; Tang et al., 2020). *Termitomyces* species are known for their taste and texture, and locally famed as Jizong (chicken-mushroom), or Yizong meaning ant associated mushrooms (Zang, 1981; Wang and Liu, 2002; Shi et al., 2012; Tang and Yang, 2014). Several species of the genus contain a high nutrient value on dry weight basis as crude fibers 17.5–24.7 g/100 g; lipids 2.5–5.4 g/100 g and proteins 15.1–19.1 g/100 g, enzymes like amylases, xylanases and cellulase, antioxidants like polyphenols (Kansci et al., 2003; Oyetayo, 2012). An estimate of 34 species representing world-wide distribution of the genus has been reported by He et al., 2019, but according to the Index Fungorum[1], genus *Termitomyces* includes 38 validated species. Only seven species of the genus have been previously reported from Pakistan (Table) (Ahmad et al., 1997; Sultan et al., 2001; Sultana et al., 2011; Sultana et al., 2014; Hussain et al., 2015). Ongoing work on the diversity of macrofungi of Pakistan, a new species erected in this study, has been delineated using morphological characteristics and molecular tools via internal transcribed spacer (ITS) region as well as large subunit (LSU) of the nuclear ribosomal DNA (nrDNA).

2. Materials and methods

2.1. Sampling protocols

During mushroom sampling from July to August, 2017–2018 in district Sheikhupura, Punjab, Pakistan, an undescribed species of *Termitomyces* was collected from a grassland area along Hiran Minar located three miles away

[1] Index Fungorum (2020). Index Fungorum [online]. Website www.indexfungorum.org [accessed 27 July 2020].

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Table. Comparison of the diagnostic characteristics of *T. sheikhupurensis* with other species reported from Pakistan.

Characters	*T. sheikhupurensis*	*T. clypeatus*	*T. eurhizus*	*T. latestui*	*T. microcarpus*	*T. striatus* f. *annulatus*	*T. umkowaan*
Pileus	Conical to plano-convex, light brownish grey, dull orange near margins	Convex, greyish brown	Convex, umber brown at apex, whitish elsewhere	Campanulate to convex later fully expanded, creamy surface, brown at umbo	Conico-campanulate, convex, getting umbrella like at maturity,white to grayish	Conical, later convexo-applanate, brownish	Campanulate to ovate, later shallowlystar shaped, pale greenish yellow topale brown
Perforatorium	Nipple-shaped	Spiniform	Broadly umbonate	Blunt cylindrical	Conical umbo	Pointed	Papillate
Annulus	Annulus absent	No membranous annulus present	Annulate or not	Annulate	With or without a minute ring	Annulus present	Annulus absent
Spores	4.7–8.52 × 3.2–6.2 µm	4.8–7 × 3.2–4 µm	6–8.5 × 3.5–5 µm	6–10 × 3–4 µm	6–7 × 4.5 µm	5–6.7 × 3.3–4.5 µm	5.9–8.8 × 3.3–5.0 µm
Cystidia	Polymorphic, clavate some with median constrictions, broadly clavate, utriform, narrowly utriform, lageniform, pyriform sometimes rostrate	Pyriform to inflated fusiform	Pyriform with a mucronate apex	Pyriform and broadly clavate	Pyriform, clavate or cylindrical	Pyriform, clavate or cylindric with a broad obtuse apex which is sometimes truncate	No cheilocystidia, pleurocystidia clavate to elongated
Habit and Habitat	Emerging from honeycomb pieces thrown out by white ants, Hiran Minar, Sheikhupura,	On termite nest, Lahore, Shakar Parian, Islamabad.	On termite nest, Kharian, Daphar Plantations, Lahore, Wah Cantt. Islamabad.	On termite nest, Ghakkar.	Arising from honeycomb fragments thrown out by white ants, Ladhar, Sheikhupura, Lahore, Ganda Singh Wala, Changa Manga, Rasul Forest, Sarai Alamgir, Islamabad, Jand-Fatehgarh, Kohat.	Nest of white ants, Jand, Islamabad	Sandy soil, Swat-Malakand Highway.

from the city, Sheikhupura. The district Sheikhupura was named because of a historical fort constructed by Mughal Emperor Noor-ud-Din Muhammad Jahangir, whose nick name was Sheikhu. He was a great lover of wildlife, when one of his pet deer named Mansraj died, a minaret and a tomb named as Hiran Minar, was built in the hunting complex, lying on the north of district (Deshmukh, 2015). The district is spread over an area of 3241 km^2 (Zahoor et al., 2017). The overall climate of the district is moist subhumid, monsoon appears from mid of June to end of August, average rainfall 630 mm and annual precipitation ranges from 250–500 mm (Nawaz et al., 2017; Shaheen et al., 2019). The soil type is mixed loamy textured and missies[2]. Predominantly, the vegetation of the district includes *Dalbergia sissoo* Roxb., *Acacia arabica* (Lam) Willd., *Abies religiosa* (Kunth) Schltdl. & Cham., *Ficus benghalensis* L. and *Albizia lebbeck* (L.) Benth. (Kazi, 1961; Zahoor et al., 2017).

2.2. Morphological protocols

Fresh fruit bodies were observed, photographed and field notes were prepared. Color codes were followed by Munsell color chart, 1975.Tissue samples (5–10 mg) were taken in 2 % CTAB buffer for DNA analyses and specimens were dried overnight in front a fan heater (EcoStar GEH-800) at 45–60 ℃. Micromorphology of the specimen was studied with the help of bright field microscope (CXRII, Labomed, Labo America Inc., Fremont, CA, USA). To examine micromorphology, 5% KOH (potassium hydroxide) solution, 1% Congo red stain and Melzer`s reagent were used. Measurements of basidiospores were done under oil immersion at 1000× magnification. To record the size of basidiospores, 80 basidiospores were measured from four fruiting bodies of two collections. Dimensions of basidiospores are given as length × width. The extreme values are given in parentheses. Other abbreviations include avX = mean of length/width between the specimens under examination, Qm (the mean of Q coefficient) = (length/width ratio), n = total number of spores measured by all collections.

2.3. Molecular protocol

The fungal DNA was extracted using the modified CTAB method. The isolated DNA was amplified using polymerase chain reaction by a thermocycler (Bio-Rad T100™, Bio-Rad Laboratories Pte. Ltd., Jurong East, Singapore). The whole internal transcribed region of nuclear ribosomal DNA (nrDNA) was amplified using the set of primers ITS1F/ITS4 and LR0R/LR5 primers for nLSU (Gardes and Bruns, 1993; Vilgalys and Hester, 1990: White et al., 1990). For sequencing, the PCR products were sent to TsingKE, China. Consensus sequences were generated by assembling both forward and reverse primer reads by BioEdit v. 7.2.5 (Hall, 1990).

Consensus sequences were subjected to BLAST (basic local alignment search tool) search in the GenBank database. Sequences showing closest identity with our newly generated sequences were selected, other with low percentage identity, poor query cover and incomplete or short sequences were omitted. To draw a phylogenetic comparison between already reported taxa based on ITS region, work of Ye et al., 2019 and for LSU region phylogenetic work of Mossebo et al., 2017 was recovered. All the available, reliable sequences of different *Termitomyces* species were retrieved from GenBank and included in the final data set along with newly generated sequences from Pakistan. Sequences of *Lyophyllum connatum* (Schumach.) (HE819396) and *Lyophyllum infumatum* (Bres.) Kühner (JF908334) in the ITS analysis (Figure1) where as *Lyophyllum ambustum* Fr. (Singer), (AF223214) and *Lyophyllum decastes* Fr. (Singer), (AF042583) were selected to root the phylogenetic tree in the LSU based analysis (Figure 2) (Sitotaw et al., 2015; Ye et al., 2019). Sequences were aligned using online alignment multiple sequence comparison by log-expectation (MUSCLE) program (Edgar, 2004) then edited manually in BioEdit (Hall, 1999). Sequence alignment was deposited in TreeBASE[3]. Maximum likelihood analyses for both gene regions was performed by CIPRES Science Gateway (Miller et al., 2010) employing RAxML-HPC v.8. For each dataset, rapid bootstrap analysis for the best-scoring maximum likelihood tree, the GTRCAT model was selected at bootstrapping phase. One thousand rapid bootstrap iterations were performed. A bootstrap value of ≥ 50% was considered significant.

3. Results

3.1. Taxonomy

***Termitomyces sheikhupurensis* Izhar, Khalid & H. Bashir sp. nov. Figures 3–5**

MycoBank no.: MB835786.

Etymology: 'sheikhupurensis' is referring to the locality of the type specimen (Hiran Minar, District Shiekhupura) from where the specimen was collected for the first time.

Diagnosis: The new species can be characterized macromorphologically by fragile fruiting bodies having velar remnants, short pseudorhiza and micromorphologically due to basidia with varying wall thickness, polymorphic cystidia and two types of pileipellis hyphae.

[2] Punjab Information & Technology Board (2020). Climate and Soil Conditions [online]. Website https://www.punjab.gov.pk/sheikhupura_climate [accessed 12 February 2020].

[3] TreeBASE (2020). A Database of Phylogenetic Knowledge [online]. Website http://purl.org/phylo/treebase/phylows/study/TB2:S26471 [accessed 00 Month Year 15 June 2020].

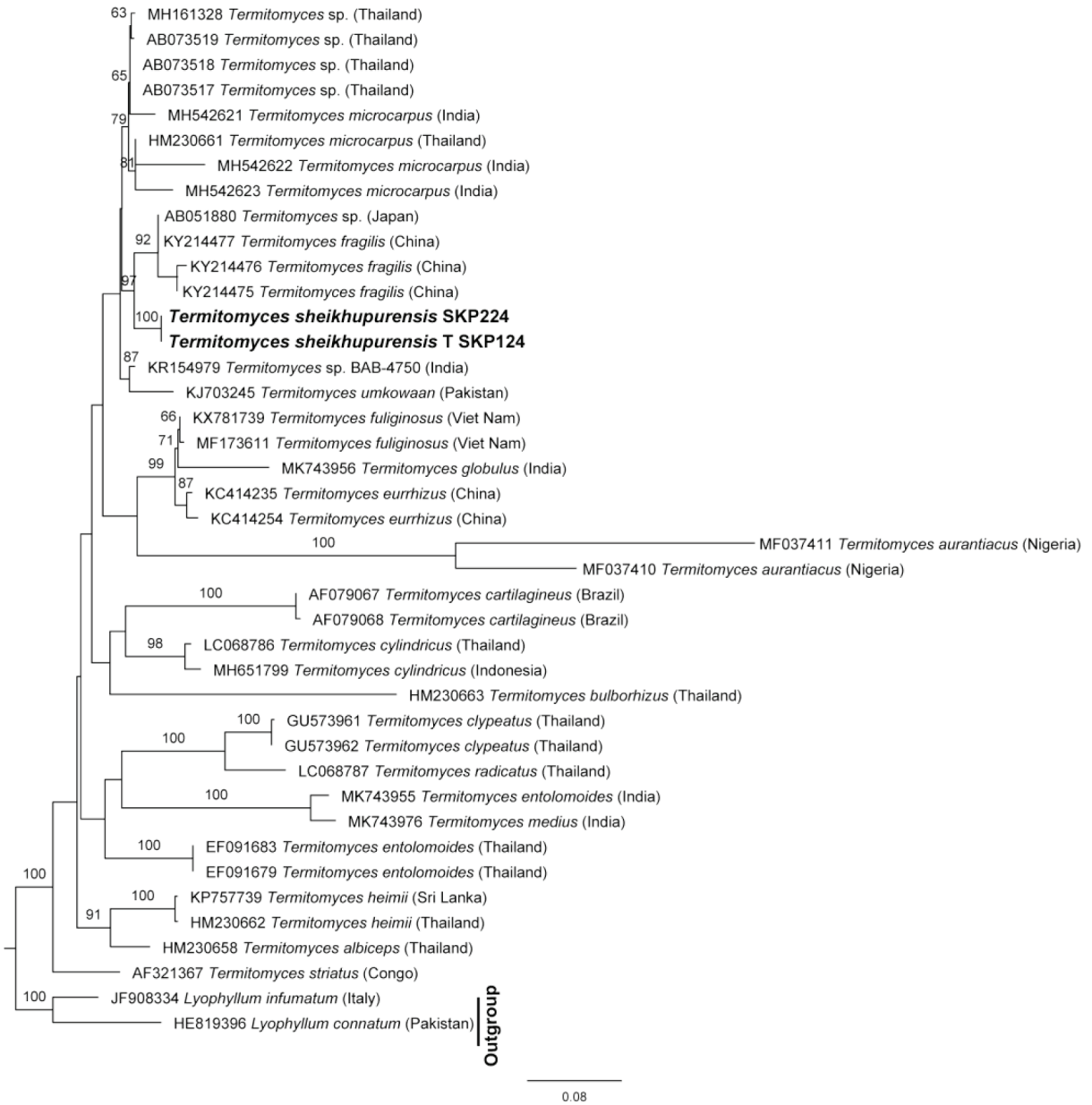

Figure 1. Maximum likelihood (ML) phylogram of *Termitomyces* drawn from the dataset of 41 ITS sequences belonging to genus *Termitomyces* and two outgroup taxa of *Lyophyllum*. Maximum likelihood bootstrap support values above 50% has been shown above the nodes. Two newly amplified sequences of *T. sheikhupurensis* are highlighted in bold.

Holotype: Pakistan, Punjab, Sheikhupura, scattered on northside of Hiran Minar in the patch of scrub like forest, emerging from honeycomb pieces thrown out by white ants, (31°44'41.75"N, 73°57'12.92″ E, 236 m a.s.l.), 02 Aug 2017, *Aiman Izhar*, *Skp124* (LAH35710), GenBank for ITS MT192217; for LSU MT192228.

Description: Pileus 1.8–3 cm diam, conical becoming plano-convex when mature, with a conspicuous nipple-shaped perforatorium measuring 0.3–0.5 cm in diameter, 0.5–0.8 cm high, surface light brownish grey (5YR7/2) at center; fading towards margin, dull orange near margins (7.5YR7/4), radially mixed with white sulcate striations, with white colored velar remnants, cuticle cracked, context whitish, thick under perforatorium, margins flaring to appendiculate, splitting radially at maturity (Figures 3A–3C). Lamellae 0.3–0.7 cm wide, dull orange (7.5YR7/4), free, regular, distant, crisped near margins, lamellulae very rare, present in 1–2 tiers (Figure 3D). Stipe 3.5–4.5 × 0.2–0.5 cm, solid, subcylindrical from upper part, tapering at base, strigose, surface pale yellow (2.58/2)

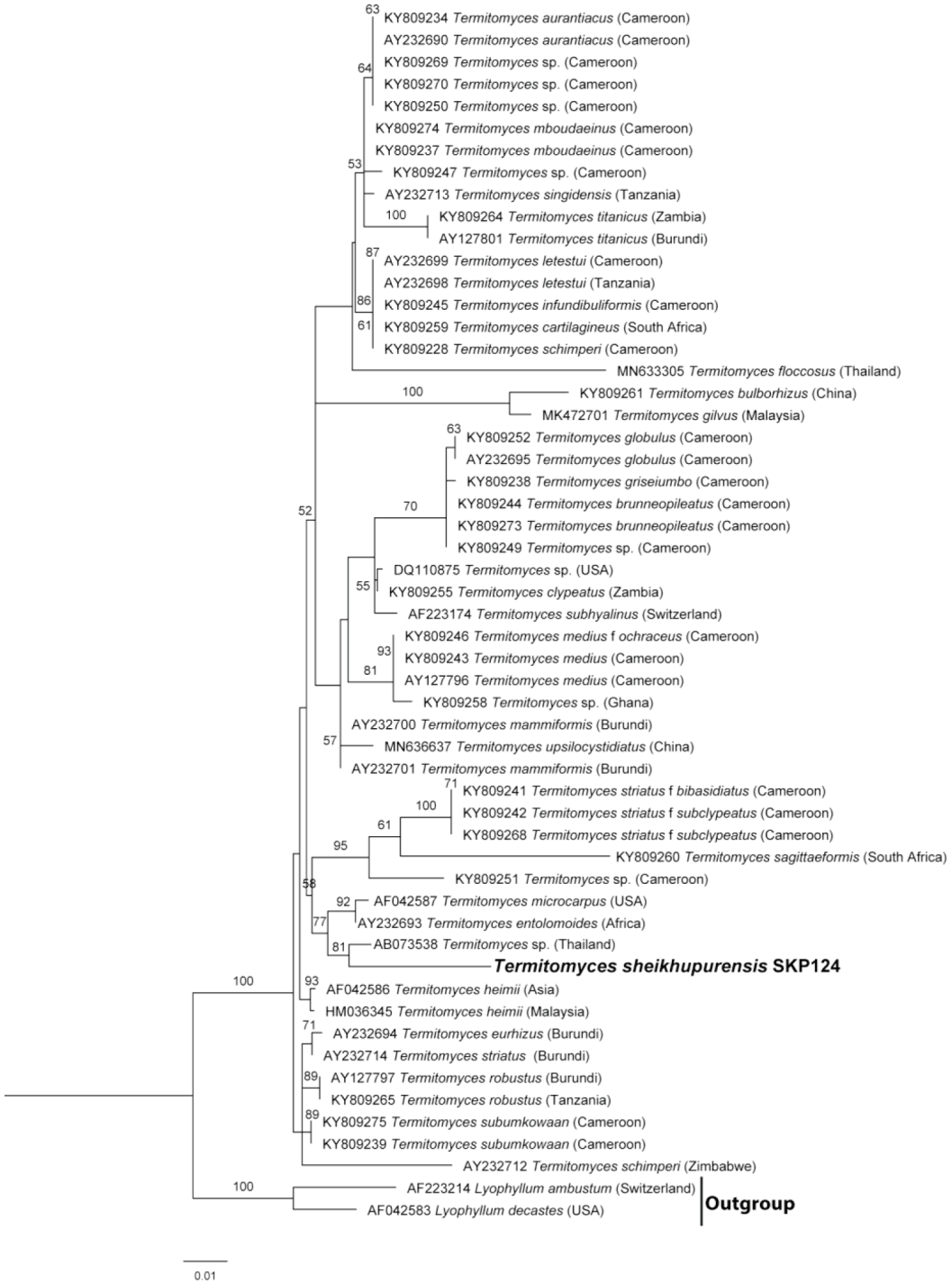

Figure 2. Molecular phylogenetic analysis by maximum likelihood (ML) method based on LSU sequences. The evolutionary history was inferred by using the RAxML-HPC2 v. 8.1.11 with a GTR + G model of nucleotide substitution. The analysis involved 56 nucleotide sequences. Evolutionary analyses were conducted in CIPRES Portal v. 3.1. Bootstrap values >50% based on 1000 replicates are shown at the branches.

near pileus, light brownish grey (5YR7/2) towards base, minutely pubescent in upper half, white striations present throughout, pseudorhiza short, 3–4 cm, rudimentary in some specimens, dark brownish (10YR 3/4) (Figures 3E, 4A, and 4B). Annulus absent. Odor not recorded. Spore print pinkish.

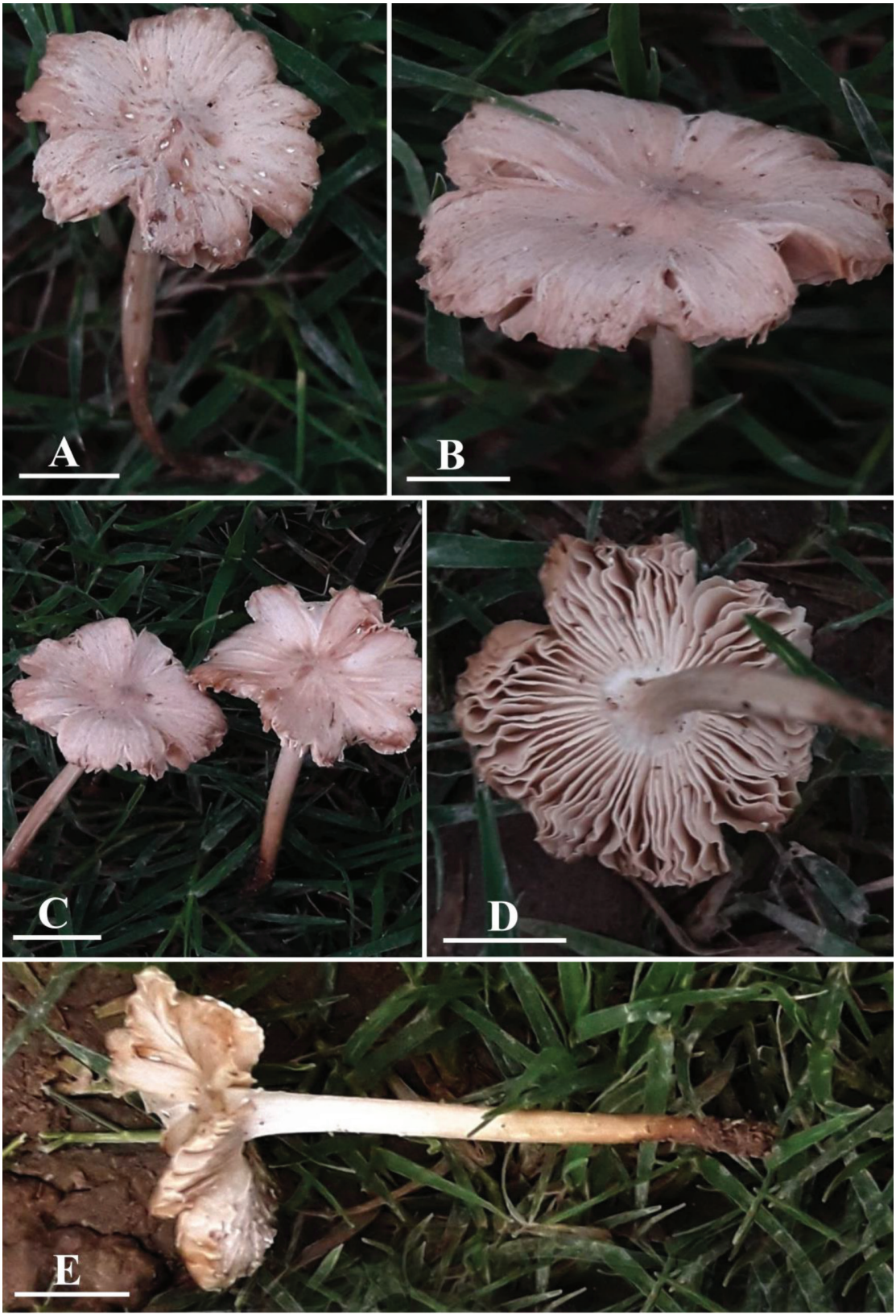

Figure 3. Macromorphological characters of *T. sheikhupurensis.* A and D = SKP124 (Holotype); B, C and E = SKP224 (Isotype). Scale Bars A–E = 1 cm, photos by Aiman Izhar.

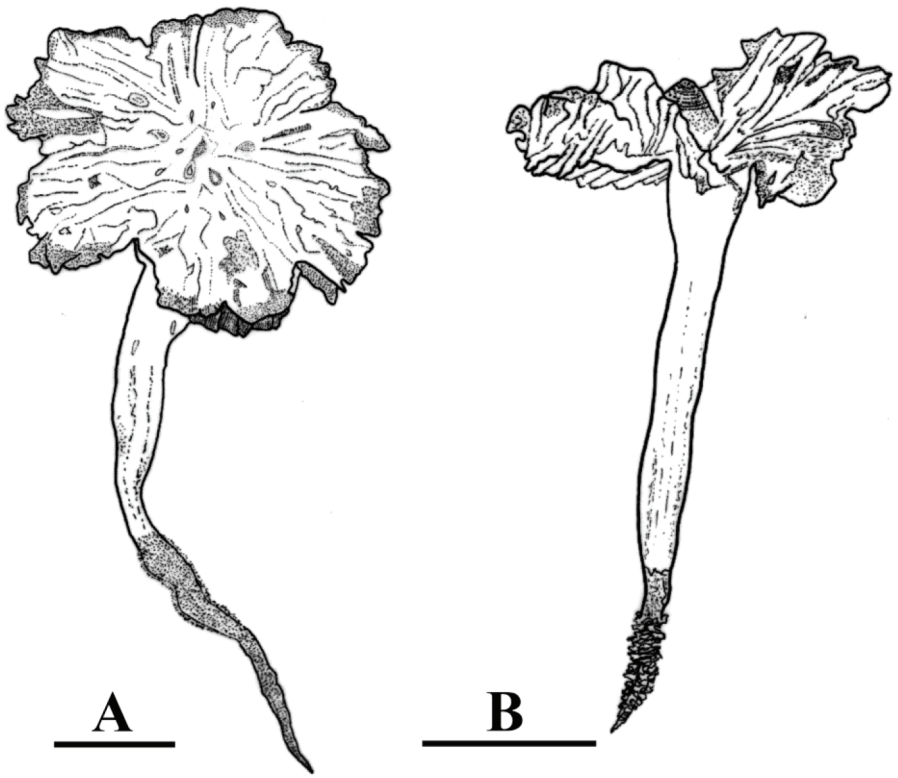

Figure 4. Illustration of the morphology of *T.sheikhupurensis,* fruiting bodies (as a schematic view). (A, B) basidiocarps with root-like pseudorhiza on stipe base. Scale Bars A, B = 1 cm, drawings by Aiman Izhar.

Basidiospores (4.7–)5.5–8.07(–8.52) × (3.2–)4.4–6.13(–6.21) µm, [avX = 6.7 ± 0.76 × 5.1 ± 0.58 µm, Qm = 1.31, n = 20 × 4], hyaline, subelliptic to ellipsoid, some subglobose, multiguttulate, thin-walled, apiculate (Figure 5A). Basidia 19–27 × 9.2–12 µm, two conspicuously different types, mainly due to thickness of membrane; one thin-walled, hyaline, clavate, second type thick-walled sclerobasidia observed, clavate to narrowly clavate, ochraceous, mostly bisporic, frequently mono- or bisporic rarely tetrasporic, (Figures 5B and 5C). Cheilocystidia 17–34 × 6–12 µm, hyaline, thin-walled, polymorphic, clavate some with median constrictions, broadly clavate, utriform, narrowly utriform, lageniform, pyriform sometimes rostrate (Figure 5D). Pleurocystidia 16–33 × 7.7–11 µm, hyaline, polymorphic, broadly clavate, sometimes showing apical septum, obpyriform, narrowly utriform to utriform, often mucronate (Figure 5E). Longitudinal section of Perforatoium 2.5–3.2 µm, upright chains of hyaline, subcylindrical, thin walled cells, few laticeferous (Figure 5F). Pileipellis composed of 2 different layers of cells, upper one having 1–3 µm, thin-walled, repent hyphae, terminal elements short cylindrical, clavate, clamp connections absent, second lower layer 16–30.4 µm, comprised of thick inflated hyphae, terminal elements clavate (Figure 5G). Stipitipellis 1.5–2.7 µm, hyaline, regular, septate hyphae, inamyloid, terminal ends swollen, 3.5–4 µm wide (Figure 5H).

Known Distribution: This species is reported for the first time from subtropical region of Punjab province, Pakistan.

Additional material examined: PAKISTAN. Punjab, Sheikhupura, found gregariously on the west side of Hiran Minar in irrigated fields, (31°44'26.58"N, 73°57'7.91"E, 236 m a.s.l.), 25 July 2018, *Aiman Izhar*, *Skp224* (LAH36413), GenBank for ITS MT192218**.**

3.2. Molecular phylogenetic analyses

Consensus sequences for the ITS region of *T. sheikhupurensis* (SKP124, SKP224) were ranging 652–654 bp after trimming. BLAST searches in the NCBI revealed that our species matches 95% to an undescribed taxon *Termitomyces* sp. (KR154979) from India and *Termitomyces* species (AB073518, AB073517, AB073519) from Thailand, recently characterized as *T. microcarpus* (Berk. & Broome) R. Heim, by Jannual et al., 2020. The position of our species in the phylogenetic tree based on ITS region (Figure 1) shows its affinities with *T. fragilis* L. Ye, Karun, J.C. Xu, K.D. Hyde & Mortimer but separates

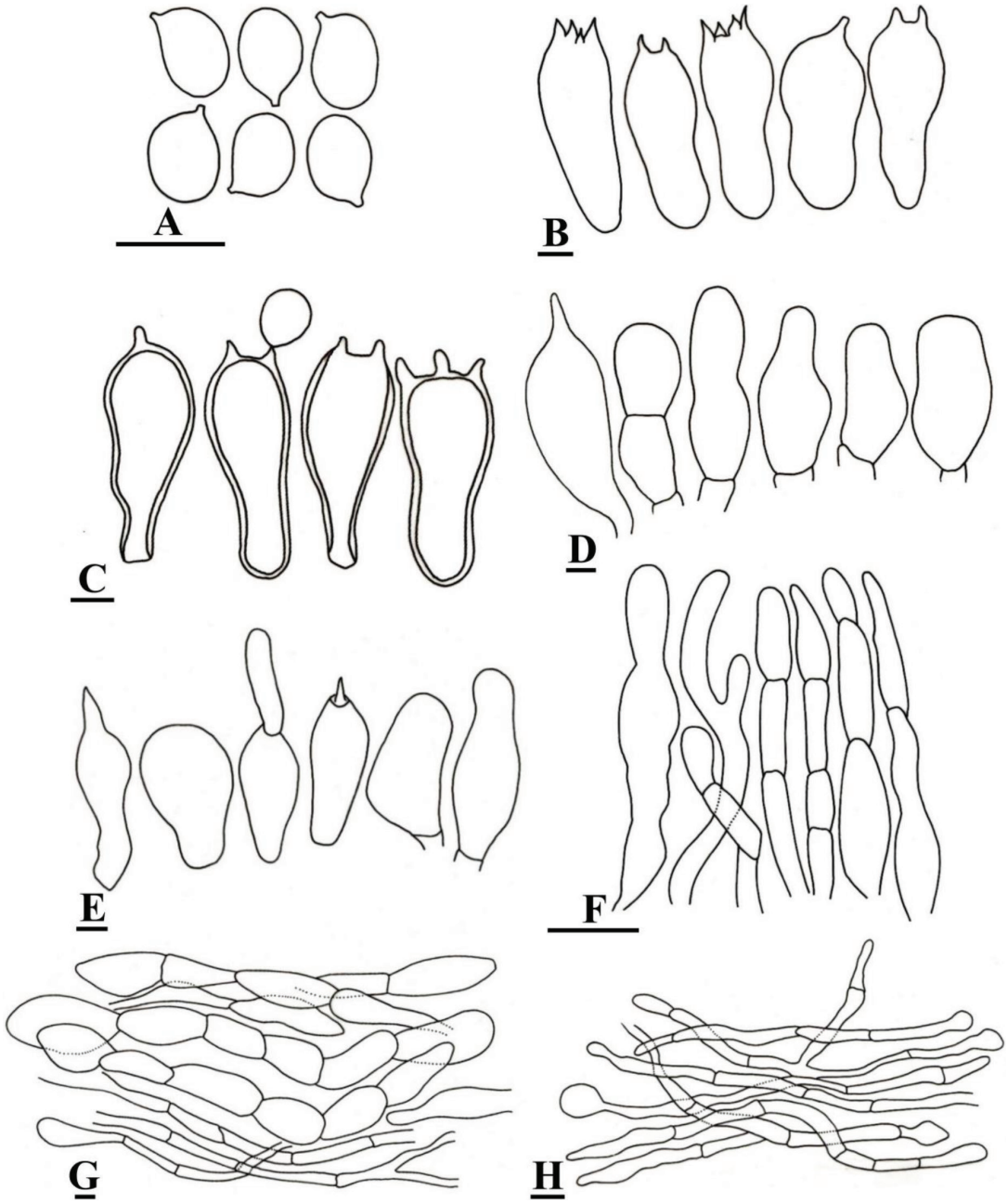

Figure 5. Micromorphological characters of *T. sheikhupurensis*. A–F (SKP124, Holotype): A = Basidiospores, B = Sclerobasidia, C = Thick-walled basidia, D = Cheilocystidia, E = Pleurocystidia, F = Longitudinal section of the perforatorium, G = Pileipellis hyphae, H = Stipitipellis hyphae. Scale Bars A–H = 5 µm, drawings by Aiman Izhar.

as a different species from *T. fragilis* by a strong bootstrap support (BS = 97%) (Figure 1).

The consensus sequence for LSU region (SKP124) was 914 bp long after trimming. The initial BLAST search with the consensus sequence of LSU region showed 98% identity with *Termitomyces* sp. (AB073538) from Thailand and *Termitomyces microcarpus* (AF042587) from USA. Our species is distinctly positioned on an isolated branch, sister to *Termitomyces* sp. (AB073538) with a strong bootstrap support(BS =81%), representing its uniqueness from other taxa used in the analyses (Figure 2).

4. Discussion

Phylogenetic analyses of the ITS sequences data showed *T. sheikhupurensis* to have a close relationship with *T. fragilis* as a sister taxon (Figure 1), recently reported from China

(Ye et al., 2019).In *T. fragilis,* the ITS sequences revealed 28 nucleotide differences incomparison to our newly proposed species. However, the LSU sequence data of *T. fragilis* is lacking so phylogenetic comparison could not be given. A single isolate of *Termitomyces* sp. BAB-4750 recorded from India, showed an identity of 95% is still an undescribed species. In LSU region based phylogram (Figure 2), sister species *Termitomyces* sp. (AB073538) giving 98% identity on BLAST analysis, reported from Thailand is still unidentified. So, no descriptions, plates, photos are available for this species, showing a lack of knowledge exists related to species of *Termitomyces*.

Macroscopically, *T. fragilis* is distinguished by having pileus surface brownish grey or greyish brown at centre, disc is comparatively darker with brownish grey, grey; reddish grey, brownish-grey, greyish-white and light grey shades, lamellae pinkish white, and lamellulae abundant. Our collections differ in having light brownish grey coloration at the centre of pileus surface, dull orange towards margin, lamellae dull orange, and lamellulae rare. Microscopic features distinguishing *T. fragilis* from *T. sheikhupurensis* are the large sized basidiospores (9 – 10.5 × 5.5–7.5 µm), thin-walled basidia, cheilocystidia and pleurocystidia much larger (40–80× 12–31 and 47–80× 14–32 µm, respectively) as described by Ye et al. (2019). Our both collections contain smaller basidiospores, thin-thick walled basidia, and comparatively much smaller cheilo- and pleurocystidia.

Termitomyces sheikhupurensis is demarcated morphologically from its closely related species *T. microcarpus* which is widely distributed in central to Southern Africa, South and Southeast Asia. *Termitomyces microcarpus* contrasts to our collections having pileus covering with straight margins and absence of velar remnants, lamellae white with crowded lamellulae, and stipe is smooth with abrupt base. Pakistani specimens possess pileus with appendiculate and radially ruptured margins with white coloured velar remnants, lamellae dull orange with lamellulae rarely present and, stipe is strigose with tapered base.

Termitomyces microcarpus, differs micromorphologically from *T.sheikhupurensis* by having ovoid to ellipsoid basidiospores, thin walled basidia, cheilocystidia and pleurocystidia are comparatively much larger reaching up to 48 and 44 µm in length, respectively, and pileipellis having narrow repent hyphae (Wei et al., 2009; Sitotaw et al., 2015).Whereas, *T. sheikhupurensis* has subelliptic, ellipsoid some subglobose basidiospores, basidia of distinctly two types; thin walled as well as thick walled sclerobasidia, cheilocystidia and pleurocystidia smaller in size reaching up to 34 and 33 µmin length, respectively, and pileipellis with two hyphal layers, one with narrower and inner with inflated elements.

If we look at the ITS tree, it is apparently evident that taxa segregated based on their stature like species having small basidiocarp structure cluster in a clade and on the contrary, we can see that species with larger size cluster in different clades. Only *T. umkowaan* clustered with taxa having small basidiomata, very closer to our species, though this species is known to have larger basidiocarp. The reason is that *T. umkowaan* has been previously reported from Karnataka region India, found in *Acacia* forests later from subtropical, dry temperate climate having Acacia species as natural vegetation of district Malakand, Pakistan (Karun and Sridhar, 2013; Hussain et al., 2015). The regions have harsh climate with less rainfall, sandy soils and almost similar vegetation type as climatic conditions of our sampling sites.

Termitomyces umkowaan (Cooke & Massee) D.A Reid. differs from *T. sheikhupurensis* by its large diameter of pileus (8–22 cm), lobate downturned margins and brownish pink, much broader (1.2 cm), crowded, laciniate lamellae and stipe with prominent psuedorhiza. Anatomically, *T. umkowaan* delineates by larger basidia 22–34 × 8–10µm (Van der Westhuizen and Eicker, 1990).

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References

Aanen DK, Eggleton P, Rouland-Lefevre C, Guldberg-Frøslev T, Rosendahl S et al. (2002). The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proceedings of the National Academy of Sciences 99 (23): 14887-14892.

Aanen DK, Eggleton P (2005). Fungus-growing termites originated in African rain forest. Current Biology 15 (9): 851-855.

Aanen DK (2006). As you reap, so shall you sow: coupling of harvesting and inoculating stabilizes the mutualism between termites and fungi. Biology Letters 2 (2): 209-212.

Ahmad S, Iqbal SH, Khalid AN (1997). Fungi of Pakistan. Lahore, Pakistan: Sultan Ahmad Mycological Society of Pakistan.

Alasoadura SO (1966). Studies in the higher fungi of Nigeria, Macrofungi associated with termites. Nova Hedwigia 11: 387-383.

Anwar K, Sudirman LI, Nandika D (2020). Comb establishment of fungus-growing termites species Macrotermitinae (Isoptera: Termitidae) with *Termitomyces cylindricus* (Basidiomycota: Agaricales) basidiospores. Oriental Insects 1-16. doi: 10.1080/00305316.2020.1762775

Aryal HP, Budathoki U (2015). Systematics of Nepalese *Termitomyces*. Our Nature 13 (1): 31-44.

Aryal HP, Ghimire SK, Budhathoki U (2016). *Termitomyces*: new to the science. Journal of Plant Science & Research 3 (1): 148-150.

Batra LR, Batra SWT (1979). Termite-fungus mutualism. In: Batra LR (editor). Insect-Fungus Symbiosis: Nutrition, Mutualism and Commensalism. International Mycological Congress. Florida, USA: John Wiley & Sons, pp. 117-163.

Deshmukh S (2015). Mughal gardens: the epitome of Islamic vision of connecting people with environment through ex situ conservation. In: The Second International Forum of the Qur'anic Botanic Garden; Doha, Qatar.

Edgar R (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1797.

Frøslev TG, Aanen DK, Læssøe T, Rosendahl S (2003). Phylogenetic relationships of *Termitomyces* and related taxa. Mycological Research 107: 1277-1286.

Gardes M, Bruns TD (1993). ITS primers with enhanced specificity forbasidiomycetes–application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Gómes LD (1995). Una nueva specie neotropical de *Termitomyces* (Agricales: Termitomycetaceae). Revista de Biologia Tropical 42: 439-451.

Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT/7. Nucleic Acids Symposium Series 41: 95-98. doi: 10.4172/2167-0412.1000264

He MQ, Zhao RL, Hyde KD, Begero D, Kemler M et al. (2019). Notes, outline and divergence times of Basidiomycota. Fungal Diversity99: 105–367. doi: 10.1007/s13225-019-00435-4

Heim R (1942). Nouvelles études descriptives sur les agarics termitophiles d'Afrique tropicale. Nouvelles archives du Muséum d'Histoire Naturelle 6 (18): 1-60.

Heim R (1977). Termites et Champignons. Paris, France: Société Nouvelle Des Éditions Boubée, pp. 1-206.

Hussain S, Afshan N, Ahmad H, Khalid AN (2015). A new report of edible mushroom, *Termitomyces umkowaan* from Pakistan. Sylwan 159 (6): 185-197.

Jannual N, Kongthong P, Nipitwatthanaphon M, Kaewrachang T, Hasin S (2020). Identification of *Termitomyces microcarpus* and termite cultures. Thai Science and Technology Journal 1281-1293. (In Thai)

Kansci G, Mossebo DC, Selatsa AB, Fotso M (2003). Nutrient content of some mushroom species of the genus *Termitomyces* consumed in Cameroon. Food/Nahrung 47 (3): 213-216.

Karun NC, Sridhar KR (2013). Occurrence and distribution of *Termitomyces* (Basidiomycota, Agaricales) in the Western Ghats and on the west coast of India. Czech Mycology 65 (2): 233-254.

Kazi (1961). District Census Report Sheikhupura, Census Report of Pakistan (no. 61). Pakistan: Population Census Organization (Pakistan).

Miller MA, Pfeiffer W, Schwartz T (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees, 2010 Gateway Computing Environments Workshop (GCE). New Orleans, LA, USA: IEEE, pp. 1-8. doi: 10.1109/GCE.2010.5676129

Morris B (1986). Notes on the genus *Termitomyces* Heim in Malawi. Society of Malawi Journal 39: 40-49.

Mossebo DC, Essouman EPF, Machouart MC, Gueidan C (2017). Phylogenetic relationships, taxonomic revision and new taxa of *Termitomyces* (Lyophyllaceae, Basidiomycota) inferred from combined nLSU- and mtSSU-rDNA sequences. Phytotaxa 321 (1): 71-102.

Munsell Color Co. (1975). Munsell Soil Color Charts. Baltimore, MD, USA: Munsell Color Co.

Natarajan K (1979). South Indian Agaricales V: *Termitomyces heimii*. Mycologia 71: 853-855.

Nawaz M, Wahla AJ, Kashif MS, Waqar MQ, Ali MA et al. (2017). Effects of exogenous nitrogen levels on the yield of rice grain in Sheikhupura, Pakistan. Pakistan Journal of Agricultural Research 30 (1): 85-92.

Njouonkou AL, Ekobo SAB, Njayou FN, Raspé O, Moundipa PF et al. (2020). Occurrence, use and antioxidant potential of *Termitomyces reticulatus* in Cameroon. Czech Mycology 72 (1): 19-32.

Nobre T, Fernandes C, Boomsma JJ, Korb J, Aanen DK (2011). Farming termites determine the genetic population structure of *Termitomyces* fungal symbionts. Molecular Ecology 20 (9): 2023-2033. doi: 10.1111/j.1365-294X.2011.05064.x

Otieno NC (1964). Contributions to knowledge of Termite fungi in East Africa. Proceedings of the East African Academy 11: 108-120.

Oyetayo VO (2012). Wild *Termitomyces* species collected from Ondo and Ekiti States are more related to African species as revealed by ITS region of rDNA. The Scientific World Journal: 1-5.

Pegler DN, Rayner RW (1969). A contribution to the Agaric flora of Kenya. Kew Bulletin 23: 347-412.

Pegler DN (1977). A preliminary agaric flora of East Africa. London, UK: Her Majesty's Stationery Office, p. 615.

Pegler DN, Vanhaecke M (1994). *Termitomyces* of Southeast Asia. Kew Bulletin 49 (4): 717-736.

Sathiya Seelan, JS, Shu Yee C, She Fui F, Dawood M, Tan YS et al. (2020). New species of *Termitomyces* (Lyophyllaceae, Basidiomycota) from Sabah (Northern Borneo), Malaysia. Mycobiology 48 (2): 95-103. doi: 10.1080/12298093.2020.1738743

Shaheen A, Iqbal J, Hussain S (2019). Adaptive geospatial modeling of soil contamination by selected heavy metals in the industrial area of Sheikhupura, Pakistan. International Journal of Environmental Science and Technology 16 (8): 4447-4464.

Sultan A, Nazir KISHWAR, Ayub N (2001). Some common species of fleshy macromycetes (Discomycetes, Gasteromycetes and Agaricales) from Margalla hills national park and adjacent areas. Pakistan Journal of Botany 33: 709-721.

Sultana K, Rauf CA, Riaz N, Farah N, Irshad G et al. (2011). Checklist of agarics of Kaghan valley-1. Pakistan Journal of Botany 43 (3): 1777-1787.

Sultana K, Riaz N, Irshad G, Khan AN (2014). Research note: contribution to mushroom flora of Rawalpindi-Islamabad, Pakistan. Journal of Bioresource Management 1 (1): 2.

Shi MX, Zhu LI, Yang KY (2012). Analysis on the nutritional components in different parts of *Termitomyces albuminosus*. Food Research and Development 33: 182-183.

Sitotaw R, Mulat A, Abate D (2015). Morphological and molecular studies on Termitomyces species of Menge District, Asossa Zone, Northwest Ethiopia. Science, Technology and Arts Research Journal 4 (4): 49-57.

Tang LP, Yang ZL (2014). Advances in fungal resources of the Lancang River-Mekong River Basin. Resources Science 36: 282-295.

Tang SM, He MQ, Raspe O, Luo X, Zhang XL et al. (2020). Two new species of *Termitomyces* (Agaricales, Lyophyllaceae) from China and Thailand. Phytotaxa 439 (3): 231-242. doi: 10.11646/phytotaxa.439.3.5

Tibuhwa DD, Kivaisi AK, Magingo FFS (2010). Utility of the macro-micromorphological characteristics used in classifying the species of *Termitomyces*. Tanzania Journal of Science 36: 3-46.

Van der Westhuizen, GCA Eicker A (1990). Species of *Termitomyces* occurring in South Africa. Mycological Research 94: 923-937.

Wang X, Liu P (2002). Resources investigation and studies on the wild commercial fungi in Yunnan. Biodiversity Science 10: 318-325.

Wei TZ, Yao YJ, Li TH (2003). First record of *Termitomyces entolomoides* in China. Mycotaxon 88: 433-438.

Wei TZ, Tang BH, Yao YJ (2009). Revision of *Termitomyces* in China. Mycotaxon 108 (1): 257-285.

White TJ, Bruns TD, Taylor LJ (1990). Amplification direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelf DH, Sninsky JJ, White TJ (editors). PCR Protocols: A Guide to Methods Applications. New York, NY, USA: Academic Press, pp. 315-322.

Ye L, Karunarathna SC, Li H, Xu J, Hyde KD et al. (2019). A survey of *Termitomyces* (Lyophyllaceae, Agaricales), including a new species, from a subtropical forest in Xishuangbanna, China. Mycobiology 1-10.

Zahoor M, Yousaf Z, Aqsa T, Haroon M, Saleh N et al. (2017). An ethnopharmacological evaluation of Navapind and Shahpur Virkanin district Sheikhupura, Pakistan for their herbal medicines. Journal of Ethnobiology and Ethnomedicine 13 (1): 27.

Zang M (1981). Notes on the classification and distribution of *Termitomyces* from Yunnan. Acta Botanica Yunnanica 3: 367-374.